

CLAIMS

1. A biosensor comprising: an electrically insulating base plate; an electrode system including a working electrode and a counter electrode formed on said base plate; a cover member joined with said base plate to define a sample supply pathway through which a sample solution is introduced from a sample supply port to said electrode system, said sample supply pathway being formed between said cover member and said base plate; and a reagent layer formed in said sample supply pathway, wherein said reagent layer contains a cholesterol-oxidizing enzyme, cholesterol esterase, an electron mediator, and a buffer having a buffering capacity in an acidic pH range.

2. The biosensor in accordance with claim 1, wherein said buffer is selected from the group consisting of succinic acid and its salts, citric acid and its salts, phthalic acid and its salts, maleic acid and its salts, and phosphoric acid and its salts.

3. The biosensor in accordance with claim 2, wherein said buffer has a buffering capacity in a pH range of 4 to 6.5.

4. The biosensor in accordance with claim 3, wherein the amount of said buffer is 5 to 1000 nmol per sensor.

5. The biosensor in accordance with claim 4, wherein the amount of said buffer is 20 to 500 nmol per sensor.

6. The biosensor in accordance with claim 1, wherein said cholesterol-oxidizing enzyme and said cholesterol

esterase are carried separately from said electron mediator in said sample supply pathway.

7. The biosensor in accordance with claim 1, wherein said buffer is carried while being mixed with said cholesterol-oxidizing enzyme or said cholesterol esterase in said sample supply pathway.

8. The biosensor in accordance with claim 1, wherein said buffer is carried while being mixed with said electron mediator in said sample supply pathway.

9. The biosensor in accordance with claim 1, wherein said buffer is carried separately from said cholesterol-oxidizing enzyme, said cholesterol esterase and said electron mediator in said sample supply pathway, and said buffer is carried at a position closer to said sample supply port than said cholesterol-oxidizing enzyme, said cholesterol esterase and said electron mediator.

10. The biosensor in accordance with claim 1, further comprising a filter in said sample supply pathway.

11. The biosensor in accordance with claim 10, wherein said filter is disposed at a position close to said sample supply port.

12. The biosensor in accordance with claim 10, wherein said filter carries said buffer.

13. The biosensor in accordance with claim 1, wherein said cholesterol-oxidizing enzyme is cholesterol oxidase.

14. The biosensor in accordance with claim 1, which is designed for measuring a body fluid.

15. The biosensor in accordance with claim 14, wherein said body fluid is blood, plasma, lymph or interstitial fluid.

16. A measuring system including:

(a) a biosensor comprising: an electrically insulating base plate; an electrode system including a working electrode and a counter electrode formed on said base plate; a cover member joined with said base plate to define a sample supply pathway through which a sample solution is introduced from a sample supply port to said electrode system, said sample supply pathway being formed between said cover member and said base plate; and a reagent layer formed in said sample supply pathway, wherein said reagent layer contains a cholesterol-oxidizing enzyme, cholesterol esterase, an electron mediator, and a buffer having a buffering capacity in an acidic pH range;

(b) voltage application means for applying a voltage between said working electrode and said counter electrode; and

(c) current detection means for detecting a current flowing between said working electrode and said counter electrode upon application of the voltage.

17. The measuring system in accordance with claim 16, further comprising a display unit for displaying a current detected by said current detection means or a converted value

of said current.

18. A method of substrate quantification using a biosensor comprising: an electrically insulating base plate; an electrode system including a working electrode and a counter electrode formed on said base plate; a cover member joined with said base plate to define a sample supply pathway through which a sample solution is introduced from a sample supply port to said electrode system, said sample supply pathway being formed between said cover member and said base plate; and a reagent layer formed in said sample supply pathway, wherein said reagent layer contains a cholesterol-oxidizing enzyme, cholesterol esterase and an electron mediator,

said method comprising:

a pretreatment step of mixing a buffer having a buffering capacity in an acidic pH range with the sample solution;

a step of supplying the sample solution subjected to said pretreatment step to said biosensor; and

a step of determining a substrate contained in said sample solution in said biosensor.

19. An enzyme reagent comprising a buffer having a buffering capacity in an acidic pH range and enzymes, wherein said enzymes are at least a cholesterol-oxidizing enzyme and cholesterol esterase.

20. The enzyme reagent in accordance with claim 19,

wherein said buffer is selected from the group consisting of succinic acid and its salts, citric acid and its salts, phthalic acid and its salts, maleic acid and its salts, and phosphoric acid and its salts.

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